

# Genetic characterization of *Allium tuncelianum*: An endemic edible *Allium* species with garlic odor

Meryem Ipek <sup>a,\*</sup>, Ahmet Ipek <sup>b</sup>, Philipp W. Simon <sup>c</sup>

<sup>a</sup> Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Horticulture, Çanakkale 17020, Turkey

<sup>b</sup> Uludağ University, Faculty of Agriculture, Horticulture Department, Görükle, Bursa 16059, Turkey

<sup>c</sup> University of Wisconsin, Department of Horticulture and U.S. Department of Agriculture-Agricultural Research Service, Vegetable Crops Research Unit, 1575 Linden Drive, Madison, WI 53706, USA

Received 30 June 2007; received in revised form 4 October 2007; accepted 6 November 2007

## Abstract

*Allium tuncelianum* (Kollman) Özhatay, Matthew & Şiraneci is a native species to the Eastern Anatolia. Its plant architecture resembles garlic (*Allium sativum* L.) and it has mild garlic odor and flavor. Because of these similarities between two species, *A. tuncelianum* has been locally called “garlic”. In addition, both *A. tuncelianum* and garlic has 16 chromosomes in their diploid genomes. Recently, *A. tuncelianum* has been suggested as the wild progenitor species of garlic. In this study, amplified fragment length polymorphisms (AFLP) markers and nucleotide sequence analysis of the internal transcribed spacer region (ITS) were used to assess genetic and phylogenetic relationships among *A. tuncelianum*, garlic and some other *Allium* species. AFLP analysis demonstrated that *A. tuncelianum* and garlic are genetically distinct and they are likely different species. Phylogenetic analyses based on the nucleotide sequence of ITS suggested that *A. tuncelianum* and garlic are distinct species and placed *A. tuncelianum*, garlic, *Allium ampeloprasum* and *Allium scorodoprasum* into the same clade in the neighbor joining dendrogram and in the consensus tree of parsimony analysis. However, *A. tuncelianum* was phylogenetically less related to garlic than either *A. ampeloprasum* or *A. scorodoprasum*, suggesting that *A. tuncelianum* may not be the immediate wild ancestor species of garlic. Further studies to generate hybrid progeny between *A. tuncelianum* and garlic (if possible) could provide more information on the homology between the chromosomes of *A. tuncelianum* and garlic and genetic relationships between these two species.

© 2007 Elsevier B.V. All rights reserved.

**Keywords:** AFLP; ITS; Phylogeny; *Allium sativum*; Ancestor species

## 1. Introduction

Garlic (*Allium sativum* L.) has been cultivated since the ancient times and its progenitor species has been suggested but not yet identified. Fritsch and Friesen (2002) have suggested that if there is a wild ancestor species of garlic, it should grow in the region from Mediterranean to south Central Asia, based on their taxonomic studies. A wild garlic relative from central Asia, *Allium longicuspis*, was proposed as a progenitor species of garlic (Vvedensky, 1944). However, recent studies based on the DNA markers demonstrated that *A. longicuspis* is not a distinct

species from common garlic (Maass and Klaas, 1995; Al-Zahim et al., 1997; Ipek et al., 2003). Mathew (1996) suggested that *Allium tuncelianum* which is utilized as garlic in Eastern region of Turkey might be the wild ancestor of garlic. The plants of three species, *A. sativum*, *A. longicuspis*, and *A. tuncelianum* share some common characteristics such as odor, coiling of the flower stem before anthesis, pale colored, small, glabrous, rather narrow perianth segments, and glabrous filaments with very long lateral cusps (Mathew, 1996; Etoh and Simon, 2002).

*A. tuncelianum* is originally named as *Allium macrochaetum* Boiss and Haussk subsp. *tuncelianum* Kollmann (Etoh and Simon, 2002). Although, it is native to ‘Tunceli’ province (especially at Platos of Munzur Mountains in Ovacik district) of Turkey, it naturally grows in the limited region located between Sivas and Erzurum provinces (Baktir, 2005). Due to its resemblance to common garlic, it is locally called as ‘Tunceli garlic’ or ‘Ovacik garlic’ in the region.

\* Corresponding author. Çanakkale Onsekiz Mart Üniversitesi, Ziraat fakültesi, Bahçe Bitkileri Bölümü, 17020 Çanakkale, Turkey.  
Tel.: +90 286 2180018x1309; fax: +90 286 2180545.

E-mail address: [msipek@comu.edu.tr](mailto:msipek@comu.edu.tr) (M. Ipek).

*A. tuncelianum* usually forms single cloved white bulb, unlike garlic which has a multiple cloved bulb. The flower scape of *A. tuncelianum* coils early in its elongation, which is a typical characteristic of some garlic types. While *A. tuncelianum* forms non-bulbiferous inflorescences with fertile flowers, all flowering garlic genotypes have bulbils formation in their inflorescences along with the flowers. Bulbil formation in the garlic inflorescence has been suggested as a cause of garlic sterility (Koul and Gohil, 1970), but this has been refuted by studies generating true seed (reviewed by Simon and Jenderek, 2003). Cytological studies on *A. tuncelianum* has demonstrated that its genome is diploid with  $2n = 16$  chromosomes, which is the same number of chromosomes with most of the widely cultivated edible *Allium* species, except with leek that has predominantly tetraploid genome (Ozhatay, 2002; Mathew, 1996). Although *A. tuncelianum* has been considered as very close relative to garlic, the exact genetic or phylogenetic relationship of this species with garlic and other similar *Allium* species is not known to our knowledge.

The internal transcribed spacer region (ITS) consisting of 5.8S ribosomal RNA gene (rDNA) and flanking internal transcribed spacer 1 and 2 (ITS-1 and ITS-2) has been successfully used to characterize the phylogenetic relationships among the species of the genus *Allium* (Dubouzet and Shinoda, 1998, 1999; Mes et al., 1999; Friesen et al., 2000; Fritsch and Friesen, 2002). The genus *Allium* consists of about 750 species and most of them naturally grow in northern hemisphere (Stearn, 1992). Controversial taxonomy of this genus has been revised and will probably continue to be revised. The genus, *Allium* has been classified under *Liliaceae*, *Amaryllidaceae*, and recently *Alliaceae* family (Mes et al., 1999). Based on nucleotide sequence of ITS of nuclear ribosomal DNA (rDNA), Fritsch and Friesen (2002) recognized 14 monophyletic subgenera in this genus. According to phylogenetic analyses, *A. sativum* L., *A. porrum* L., and *Allium ampeloprasum* L. that are morphologically similar species to *A. tuncelianum* have been classified under subgenus *Allium* section *Allium* (Hanelt, 1990; Mes et al., 1999; Ricroch et al., 2005). In this respect, the purpose of this study was to investigate phylogenetic and genetic relationships of *A. tuncelianum* with garlic and other related *Allium* species using amplified fragment length polymorphisms (AFLP) and the sequence of ITS region.

## 2. Materials and methods

### 2.1. Plant materials

Two different accessions of *A. tuncelianum*, four accessions of garlic, one accession of *A. longiscuspis* and 17 other *Allium* species were included to this study (Table 1). *A. tuncelianum* accessions were obtained from Tunceli province of Turkey. Other *Allium* species except *Allium cepa* were obtained from US Department of Agriculture, Western Region Plant Introduction Station, Pullman, WA, USA. Seeds of *Allium* species were planted to pots in the Walnut Street, Greenhouse at University of Wisconsin, Madison, WI, USA, and young leaves were sampled from single plant of each accession. Accessions

Table 1

Accession numbers and the length of ITS regions in the *Allium* species analyzed in this study

Accession number	Species	Length of ITS (bp)
PI592999	<i>A. roylei</i>	641
W612754	<i>A. oschaninii</i>	642
W612755	<i>A. pskemense</i>	627
PI280549	<i>A. altaicum</i>	643
PI219754	<i>A. fistulosum</i>	643
PI576875	<i>A. altynolicum</i>	632
PI371880	<i>A. schoenoprasum</i>	632
W621059	<i>A. lineare</i>	641
PI576906	<i>A. hymenorrhizum</i>	641
W618966	<i>A. polyrhizum</i>	633
PI369526	<i>A. senescens</i>	643
W614000	<i>A. ampeloprasum</i>	642
PI576936	<i>A. scorodoprasum</i>	642
PI576926	<i>A. ramosum</i>	646
PI576957	<i>A. tuberosum</i>	645
W620304	<i>A. cernuum</i>	655
gi:216278871	<i>A. cepa</i>	639
Turkey	<i>A. tuncelianum</i> 1	643
Turkey	<i>A. tuncelianum</i> 2	643
U094-4	<i>A. longiscuspis</i>	642
U074	<i>A. sativum</i>	642
PI515971	<i>A. sativum</i>	642
PI383817	<i>A. sativum</i>	642
PI497949	<i>A. sativum</i>	642

of *A. tuncelianum*, *A. longiscuspis* and garlic were also planted in a field in Bursa province of Turkey to observe the morphology of these species. ITS sequence of *A. cepa* (gi:216278871) were obtained from the National Center for Biotechnology Information (NCBI) GenBank databases (<http://www.ncbi.nlm.nih.gov>) and included to phylogenetic analysis.

### 2.2. DNA extraction and PCR procedures

Lyophilized leaf samples were powdered using a paint shaker and approximately 150 mg of powder obtained were transferred to micro centrifuge tubes for DNA extraction. DNA samples were extracted according a modified CTAB method described by Fütterer et al. (1995). ITS regions (ITS-1, 5.8S rDNA subunit, ITS-2) were amplified using primer combination of ITSA and ITSB (Blattner, 1999). Each 25  $\mu$ L polymerase chain reaction (PCR) consisted of 0.75 Units of DNA polymerase (PanVera, Madison, WI, USA) with the reaction buffer supplied at  $1\times$  concentration, 0.8  $\mu$ M of each primer, dNTPs at 200  $\mu$ M each, and 40–60 ng template DNA. The reactions were heated to 94 °C for 2 min followed by 35 cycles of 95 °C for 20 s, 55 °C for 45 s, and 68 °C for 1 min and final extension of 72 °C for 5 min. For these reactions, a Perkin Elmer model 9600 Thermal Cycler was used. PCR products were size fractionated by electrophoresing through 1.5% (w/v) agarose in  $1\times$  TAE buffer (40 mM Tris-acetate, pH 8.0 and 1 mM EDTA) with a GeneRuler™ 100 bp DNA ladder (Fermentas, Hanover, MD, USA) as DNA molecular weight marker. Gels were stained with ethidium bromide (0.5  $\mu$ g mL<sup>-1</sup>) (Sigma, St Louis, MO, USA) and photographed.

### 2.3. Sequencing of ITS region

Purification of PCR products from the agarose gel, cloning and sequencing of them were carried out according to procedures previously described by Ipek et al. (2005) except for plasmid DNA extraction procedures. Three to four bacterial colonies from each purified and cloned PCR product were picked up and subjected to PCR amplification using T7 and SP6 universal primers of cloning vector. Each PCR reaction contained the same reaction mixture described above and a single bacterial colony as a DNA template. PCR reaction conditions were 94 °C for 2 min, then 35 cycles of 95 °C for 20 s, 50 °C for 1 min, 68 °C for 2 min and a final extension of 72 °C for 5 min. To remove single stranded DNA fragment such as primers, PCR products were treated with ExoSap (USB, Cleveland, OH, USA) by following the manufacturer's protocol and 2 µl of each ExoSap treated PCR products was used for sequencing reaction.

### 2.4. Data analyses

Sequences obtained were manually edited with CHROMAS v.2.31 (Technelysium Pty. Ltd.). All sequences were aligned using CLUSTAL W option in BioEdit sequence alignment editor (Hall, 1999). No manual adjustment of alignment was made to avoid introducing subjective bias.

Aligned ITS sequences were evaluated with bootstrap analysis 2000× and Kimura (1980) distances were calculated to construct a neighbor joining (NJ) dendrogram using TREECON v. 1.3b program (van de Peer and de Wachter, 1994) with the program defaults. The NJ dendrogram was visualized by using TREECON program. The first 100 most parsimonious trees were generated by DNAPENNY in PHYLIP v. 3.6 (Felsenstein, 2002) utilizing sophisticated “branch and bound” algorithm and using the program defaults. A consensus tree of the first 100 most parsimonious trees was calculated by CONSENSE in

PHYLIP v. 3.6 using extended majority rule option. During construction of the NJ tree and calculation of most parsimonious trees, ITS nucleotide sequences of *Nothoscordum bivalve* (gi:11595756), which was obtained from NCBI GenBank was included into analysis for rooting trees as it was suggested by Dubouzet and Shinoda (1999).

### 2.5. AFLP analysis

AFLP analysis was performed according to procedures described by Vos et al. (1995) with the modification of Ipek et al. (2003). EACGG/MCTC primer combination was used to compare the AFLP band profiles of *A. tuncelianum* and garlic.

## 3. Results

### 3.1. Plant morphology

Plant architecture and leaf structure of *A. tuncelianum* resembled the garlic in the field in 2006 (Fig. 1A). The accessions of *A. tuncelianum* had coiling of their scapes before anthesis, like garlic (Fig. 1A). Although, the accessions of both garlic and *A. tuncelianum* were planted to field at the same time (November, 2005 in Bursa, Turkey), *A. tuncelianum* started bolting much earlier than garlic (Fig. 1A,C). Flowering time of both species should be synchronized or pollen should be stored in future studies if an attempt to obtain hybrid progeny between *A. tuncelianum* and garlic is desired. During anthesis, *A. tuncelianum* had pinkish-purple fertile flowers with well-exserted filaments and styles, but the umbels did not contain any bulbils which are present in the umbels of all flowering garlic accessions, along with flowers (Fig. 1B; Etoh and Simon, 2002; Simon and Jenderek, 2003). The flowers in the umbels of *A. tuncelianum* set fertile black seeds, like other *Allium* species. *A. tuncelianum* had single bulb with a single large round clove in contrast to garlic clones which has bulbs composed of 10–20



Fig. 1. *A. tuncelianum* growing in the field. Panel A is a bolting *A. tuncelianum* with twisting flower scape on May 11, 2006. Panel B is an umbel of *A. tuncelianum* during full anthesis. Panel C is an *A. tuncelianum* during anthesis and bolting *A. sativum* plants on June 3, 2006 demonstrating the differences in the flowering time.



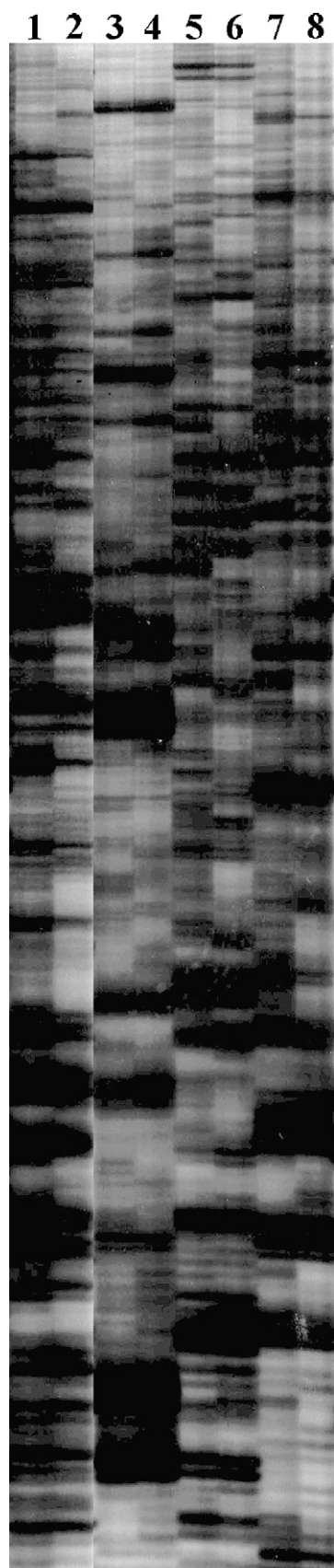


Fig. 2. Part of autoradiogram demonstrating the differences of AFLP banding profile in *A. sativum* (1, 2), *A. schoenoprasum* (3, 4), *A. ampeloprasum* (5, 6) and *A. tuncelianum* (7, 8). The AFLP banding profile is generated using the primer combination EACGG/MCTC.

cloves of relatively similar size. These observations were in agreement with the earlier description of *A. tuncelianum* (Mathew, 1996). In addition to the similarity of plant architectures and morphologies of *A. tuncelianum* and garlic, *A. tuncelianum* has mild garlic odor and flavor. Therefore, it has been called “garlic” in the region where it grows in the wild, is collected and consumed as garlic. The species is not cultivated, but it is only collected from the wild and consumed locally with increasing popularity. Because of this unconscious collection from the wild, the species faces a serious threat of extinction. In an attempt to counteract this, studies and local conservation efforts with international collaboration (a project supported by Small Grants Programme (SGP) of Global Environment Facility (GEF) of the United Nations Development Programme (UNDP)) have been initiated to take *A. tuncelianum* under cultivation and adapt it in different regions of Turkey (Yanmaz et al., 2006; [http://sgp.undp.org/web/projects/8149/promotion\\_of\\_cultivation\\_and\\_conservation\\_of\\_tunceli\\_garlic\\_allium\\_tuncelianum\\_project\\_phase\\_ii.html](http://sgp.undp.org/web/projects/8149/promotion_of_cultivation_and_conservation_of_tunceli_garlic_allium_tuncelianum_project_phase_ii.html)).

### 3.2. AFLP analysis

DNA markers have been extensively used to analyze genetic diversity in many plant species, including garlic (Al-Zahim et al., 1997; Ipek et al., 2003; Volk et al., 2004). Studies based on isozyme, RAPD and AFLP markers did not suggest that *A. longicuspis* is a distinct species from garlic (Pooler and Simon, 1993; Maass and Klaas, 1995; Al-Zahim et al., 1997; Ipek et al., 2003). Therefore, we attempted to compare AFLP banding profile of *A. tuncelianum* with garlic accessions and found that the banding profiles of these two species were very different (Fig. 2). A limited number of AFLP markers (approx. 15–20%) shared by garlic and *A. tuncelianum* suggested that *A. tuncelianum* and garlic might be distinct species. We have demonstrated in our previous study that AFLP markers of the same size in different garlic clones do not always have same nucleotide sequence identity and homology among the AFLP bands sharing the same position on a polyacrylamide gel depends on the phylogenetic relationship in garlic (Ipek et al., 2006). Therefore, the AFLP markers shared by both *A. tuncelianum* and garlic may not have the homologous nucleotide sequences and sequence identity of these AFLP markers should be characterized to make any phylogenetic conclusion.

### 3.3. ITS sequences and phylogenetic analysis

The internal transcribed spacer region (ITS) has been used for phylogenetic analyses in the genus *Allium* (Dubouzet and Shinoda, 1998, 1999; Mes et al., 1999; Friesen et al., 2000; Fritsch and Friesen, 2002). Dubouzet and Shinoda (1999) suggested DNA sequence analysis of ITS as a useful tool for intragenic classification of *Alliums*. The ITS region in *A. tuncelianum*, garlic and some other *Allium* species were sequence-characterized in this study to analyze the phylogenetic relationship among these *Allium* species. The lengths of ITS region in the *Allium* species evaluated in this study ranged

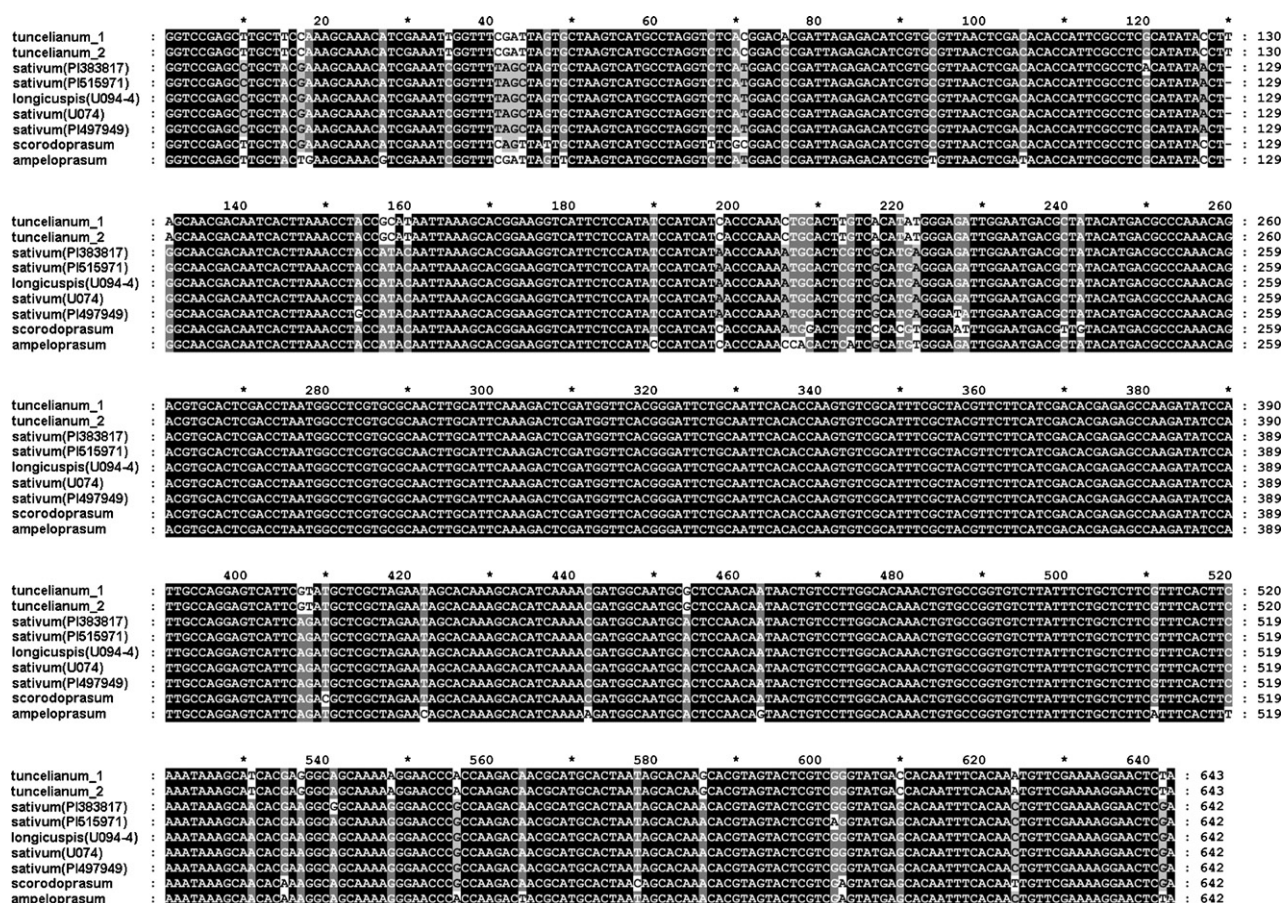


Fig. 3. Sequence alignment of ITS regions in two accessions of *A. tuncelianum*, and phylogenetically closely related *Allium* species, *A. scorodoprasum*, *A. ampeloprasum*, *A. longicuspis* and five accessions of *A. sativum*. Black, dark gray, and light gray indicate the 100%, 80%, and 60% identities, respectively. Sequence alignment was displayed using GENEDOC (Nicholas and Nicholas, 1997).

from 627 bp in *Allium pskemense* to 655 bp in *Allium cernuum* (Table 1). *A. tuncelianum* had an ITS region of 643 pb while ITS regions of garlic, *A. longicuspis*, *A. ampeloprasum* and *Allium scorodoprasum* accessions were consist of 642 bp (Fig. 3). The difference in length between the *A. tuncelianum* and garlic was due to the insertion of thymine residue at 130 bp position. In addition, *A. longicuspis* (U094-4) and a garlic accession (U074) in this study had identical ITS sequences (Fig. 3).

The ITS sequences of various *Allium* species were compared to determine the phylogenetic relationship among *A. tuncelianum* and other *Allium* species. Both the neighbor joining dendrogram and the consensus tree of parsimony analysis has the same tree topology (Fig. 4A,B). Phylogenetic relationships among *Allium* species were in agreement with previous studies (Dubouzet and Shinoda, 1999; Mes et al., 1999; Friesen et al., 2000; Fritsch and Friesen, 2002; Ricoch et al., 2005). All *Allium* species formed a monophyletic group (Fig. 4A).

The neighbor joining dendrogram and the consensus tree of parsimony analysis placed *A. tuncelianum* into the clade of subgenus *Allium* along with garlic, *A. longicuspis*, *A. ampeloprasum* and *A. scorodoprasum* (Fig. 4A,B). According to these phylogenetic dendrograms, *A. tuncelianum* separated as

species before the speciation of *A. ampeloprasum*, *A. scorodoprasum* and garlic. Therefore, our phylogenetic analysis based on the nucleotide sequences of ITS did not support the hypothesis that *A. tuncelianum* is the immediate progenitor species of garlic.

#### 4. Discussion

*A. longicuspis* has been proposed to be the ancestor species of garlic (Vvedensky, 1944). Several studies based on DNA markers demonstrated that *A. longicuspis* is not genetically distinct from garlic. Our results based on nucleotide sequence of ITS in this study also suggested that *A. longicuspis* and *A. sativum* are the same species since a garlic accession and *A. longicuspis* accession in Fig. 3 shared identical ITS sequences.

Recently, *A. tuncelianum* has been proposed as progenitor species of garlic due to the common morphological features of both species (Mathew, 1996; Etoh and Simon, 2002; Fritsch and Friesen, 2002). *A. tuncelianum* also has mild garlic odor and flavor, and it has been locally consumed as garlic in the region where it grows in the wild. Our study indicates that *A. tuncelianum* is not a likely progenitor of garlic, based upon inflorescence structure, flowering time and bulb morphology. Furthermore, our AFLP analysis revealed that *A. tuncelianum*

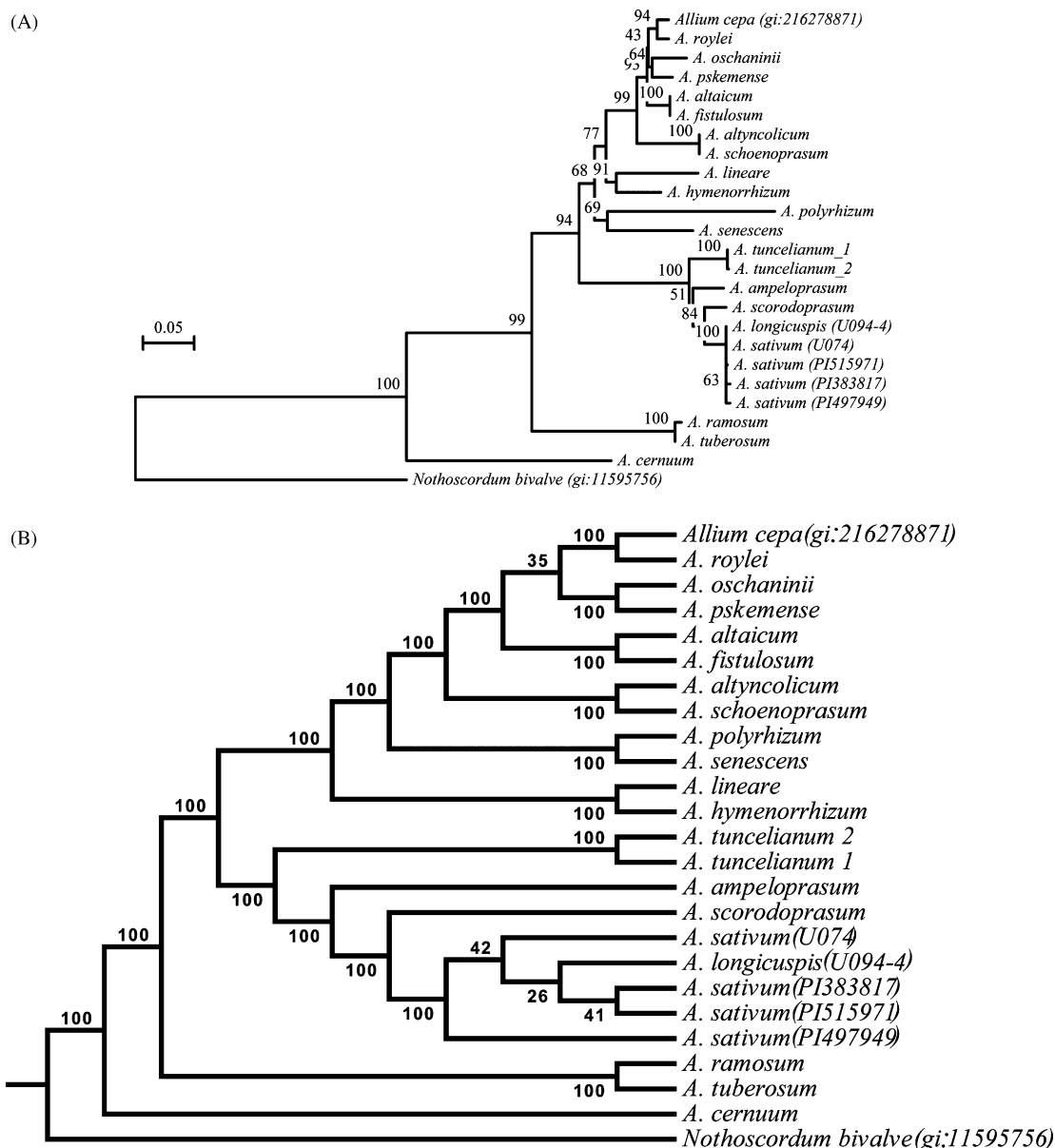


Fig. 4. Phylogeny analysis of 19 *Allium* species including two accessions of *A. tuncelianum* based on ITS sequences. Dendrogram A was generated from neighbor joining analysis from Kimura (1980) distances (TREECON, NJ). Bootstrap values are shown in percentages. Dendrogram B is the consensus dendrogram of first 100 most parsimonious tree generated by DNAPENNY (PHYLIPP). The numbers on the dendrogram indicate the number of times the group consisting of the species occurred among the first 100 most parsimonious trees.

and garlic are genetically different and they are likely distinct species. Phylogenetic analysis of ITS nucleotide sequences further supported the idea that *A. tuncelianum* is a different species from both garlic and leek. Although, *A. tuncelianum* was placed in the clade of subgenus *Allium*, *A. tuncelianum* separated before the separation of garlic, *A. ampeloprasum* and *A. scorodoprasum* as a species.

Garlic-like but odorless *Allium* species, ‘Mushuu-ninniku’, from Japan was characterized to determine its relationship with garlic and other related *Allium* species (Ariga et al., 2002). According to morphological and biochemical characteristics, karyotype, restriction fragment length polymorphism, and isozyme markers analyses, ‘Mushuu-ninniku’ was found to be more similar to leek than to garlic, although bulb structure with

multiple cloves of this plant is similar to garlic. In another study, genetic characterization of bulbous leek-like *Allium* species consumed as garlic in the Chinese diet has revealed that it was also a leek variant (Bohanec et al., 2005). Both of these recently characterized *Allium* accessions seem to be a member of subgenus *Allium*. *A. tuncelianum* could be another leek-like *Allium* species with mild garlic odor and flavor according to our results. Therefore, comparing genetic relationship of *A. tuncelianum* with these leek-like accessions could also be helpful to identify the origin of *A. tuncelianum* and to obtain a more conclusive result about the phylogeny of subgenus, *Allium*.

In conclusion, our results did not suggest *A. tuncelianum* as the immediate wild progenitor species of garlic. Further



evidence testing the suggestion of Mathew (1996) can be generated by studies on the genomic *in situ* hybridization to understand level of homology between *A. tuncelianum* and garlic chromosomes and studies to generate hybrid progeny between *A. tuncelianum* and garlic, if possible. Further phylogenetic analyses using broader diversity of *Allium* species that are native to Flora of Turkey and other molecular marker types such as plastid cpDNA can make possible to identify the origin of the *A. tuncelianum* and garlic.

## References

- Al-Zahim, M., Newbury, H.J., Ford-Lloyd, B.V., 1997. Classification of genetic variation in garlic (*Allium sativum*) revealed by RAPD. *HortScience* 32, 1102–1104.
- Ariga, T., Kumagai, H., Yoshikawa, M., Kawakami, H., Seki, T., Sakurai, H., Hasegawa, I., Etoh, T., Sumiyoshi, H., Tsuneyoshi, T., Sumi, S., Iwai, K., 2002. Garlic-like but odorless plant *A. ampeloprasum* 'Mushuu-ninniku' J. *Jpn. Soc. Hort. Sci.* 71, 362–369.
- Baktir, I., 2005. Tunceli sarımsağı'nın (*Allium tuncelianum*) *in vitro* koşullarında çoğaltılması. In: Proceeding of GAP IV. Tarım Kongresi, pp. 206–208 (in Turkish).
- Blattner, F.R., 1999. Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. *Biotechniques* 27, 1180–1186.
- Bohanec, B., Jakše, M., Šesek, P., Havey, M.J., 2005. Genetic characterization of an unknown Chinese bulbous leek-like accession and its relationship to similar *Allium* species. *HortScience* 40, 1690–1694.
- Dubouzet, J.G., Shinoda, K., 1998. Phylogeny of *Allium* L. subg. *Melanocrommyum* (Webb et Berth.) Rouy based on DNA sequence analysis of the internal transcribed spacer region of rDNA. *Theor. Appl. Genet.* 97, 541–549.
- Dubouzet, J.G., Shinoda, K., 1999. Relationships among old and new world *Alliums* according to ITS DNA sequence analysis. *Theor. Appl. Genet.* 98, 422–433.
- Etoh, T., Simon, P.W., 2002. Diversity, fertility and seed production of garlic. In: Rabinowitch, H.D., Currah, L. (Eds.), *Allium Crop Science: Recent Advances*. CABI Publishing, New York, pp. 101–117.
- Felsenstein, J., 2002. PHYLIP (Phylogeny Inference Package) version 3.6 alpha. Distributed by the author. Department of Genetics, University of Washington, Seattle, WA.
- Friesen, N., Fritsch, R.M., Pollner, S., Blattner, F.R., 2000. Molecular and morphological evidence for an origin of the aberrant genus *Milula* within Himalayan species of *Allium* (*Alliaceae*). *Mol. Phylogenet. Evol.* 17, 209–218.
- Fritsch, R.M., Friesen, N., 2002. Evolution, domestication, and taxonomy. In: Rabinowitch, H.D., Currah, L. (Eds.), *Allium Crop Science: Recent Advances*. CABI Publishing, New York, pp. 5–30.
- Fütterer, J., Gisel, A., Iglesias, V., Kloti, A., Kost, B., Mittelsten-Scheid, O., Neuhaus, G., Neuhaus-Url, G., Schrott, M., Shillito, R., Spangenberg, G., Wang, Z.Y., 1995. Standard molecular techniques for the analysis of transgenic plants. In: Potrykus, I., Spangenberg, G. (Eds.), *Gene Transfer to Plants*. Springer-Verlag, New York, pp. 215–218.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41, 95–98.
- Hanelt, P., 1990. Taxonomy, evolution and history. In: Rabinowitch, H.D., Brewster, J.L. (Eds.), *Onions and Allied Crops*, 1. CRC Press, Boca Raton, Florida, pp. 1–26.
- Ipek, M., Ipek, A., Simon, P.W., 2003. Comparison of AFLPs, RAPD markers, and isozymes for diversity assessment of garlic and detection of putative duplicates in germplasm collections. *J. Am. Soc. Hort. Sci.* 128, 246–252.
- Ipek, M., Ipek, A., Almquist, S.G., Simon, P.W., 2005. Demonstration of linkage and development of the first low-density genetic map of garlic based on AFLP markers. *Theor. Appl. Genet.* 110, 228–236.
- Ipek, M., Ipek, A., Simon, P.W., 2006. Sequence homology of polymorphic AFLP markers in garlic (*Allium sativum* L.). *Genome* 49, 1246–1255.
- Kimura, M., 1980. A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16, 111–120.
- Koul, A.K., Gohil, R.N., 1970. Causes averting sexual reproduction in *Allium sativum* Linn. *Cytologia* 35, 197–202.
- Maass, H.I., Klaas, M., 1995. Intraspecific differentiation of garlic (*Allium sativum* L.) by isozyme and RAPD markers. *Theor. Appl. Genet.* 91, 89–97.
- Mathew, B., 1996. A Review of *Allium* Section *Allium*. Royal Botanic Gardens, Kew Publishing, Richmond.
- Mes, T.H.M., Fritsch, R.M., Pollner, S., Bachmann, K., 1999. Evolution of the chloroplast genome and polymorphic ITS regions in *Allium* subgenus *Melanocrommyum*. *Genome* 42, 237–247.
- Nicholas, K.B., Nicholas, H.B. Jr., 1997. Genedoc: a tool for editing and annotating multiple sequence alignments. *Multiple Sequence Alignment and Shading Utility*, version 2.6.002. Available from <http://www.psc.edu/biomed/genedoc>. (Accessed February, 2005).
- Ozhatay, N., 2002. Diversity of bulbous monocots in Turkey with special reference. *Chromosome numbers*. *Pure Appl. Chem.* 74, 547–555.
- Pooler, M.R., Simon, P.W., 1993. Characterization and classification of isozyme and morphological variation in a diverse collection of garlic clones. *Euphytica* 68, 121–130.
- Ricroch, A., Yockteng, R., Brown, S.C., Nadot, S., 2005. Evolution of genome size across some cultivated *Allium* species. *Genome* 48, 511–520.
- Simon, P.W., Jenderek, M.M., 2003. Flowering, seed production, and the genesis of garlic breeding. *Plant Br. Rev.* 23, 211–244.
- Stearn, W.T., 1992. How many species of *Allium* are known? *Kew Mag.* 9, 180–182.
- van de Peer, Y., de Wachter, R., 1994. TREECON for windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput. Appl. Biosci.* 10, 569–570.
- Volk, G.M., Henk, A.D., Richards, C.M., 2004. Genetic diversity among US garlic clones as detected using AFLP methods. *J. Am. Soc. Hort. Sci.* 129, 559–569.
- Vos, P., Hogers, R., Bleeker, M., Reijmans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23, 4407–4414.
- Vvedensky, A.I., 1944. The genus *Allium* in the USSR. *Herbertia* 11, 65–218.
- Yanmaz, R., Besirli, G., Uzun, Y., Yazar, E., Kantoglu, Y., Alper, A., Ermis, S., 2006. Tunceli sarımsağını (*Allium tuncelianum* (Kollman) Ozhatay, Mathew, Siraneci) kültüre alma çalışmaları. In: Caglar, G., Abak, K., Akinci, I.E. (Eds.), *Proceeding of VI Sebze Tarımı Sempozyumu*. Kahramanmaraş, Turkey, (in Turkish), pp. 29–33.